Yasuda-Shedlovsky Result

Sample name: **Pyridoxine HCl**
Assay name: **UV-metric psKa**
Assay ID: **17I-12014**
Filename: **C:\Sirius_T3\Mehtap\20170912_test_comp_pKa_diffexp\17I-12014_Pyridoxine HCl_UV-metric psKa.t3r**

Experiment start time: **9/12/2017 7:57:21 PM**
Analyst: **Dorothy Levorse**
Instrument ID: **T311053**

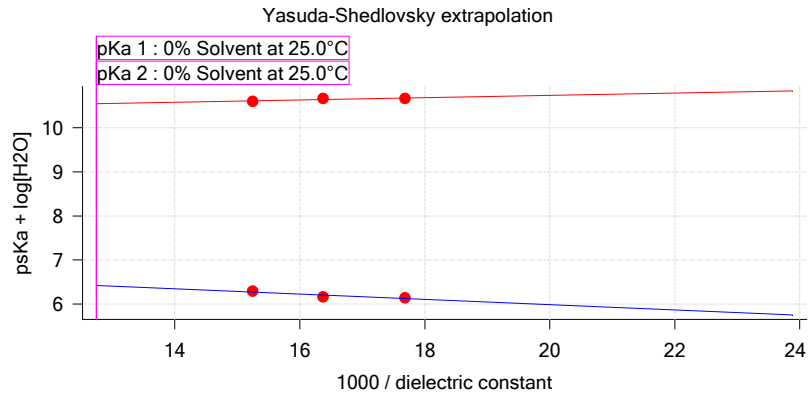
Yasuda-Shedlovsky result

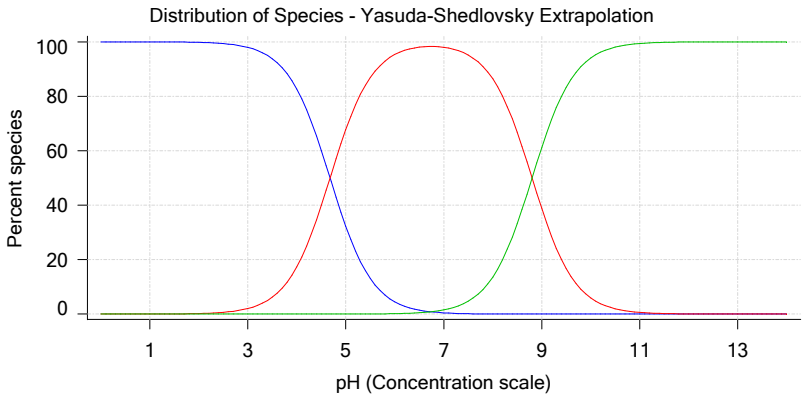
Extrapolation type	pKa 0%	SD	Intercept	Slope	R²	Ionic strength	Temperature
Yasuda-Shedlovsky	4.68	±0.10	7.19	-60.2824	0.8298	0.165 M	25.0°C
Yasuda-Shedlovsky	8.80	±0.07	10.22	25.9224	0.6711	0.165 M	25.0°C

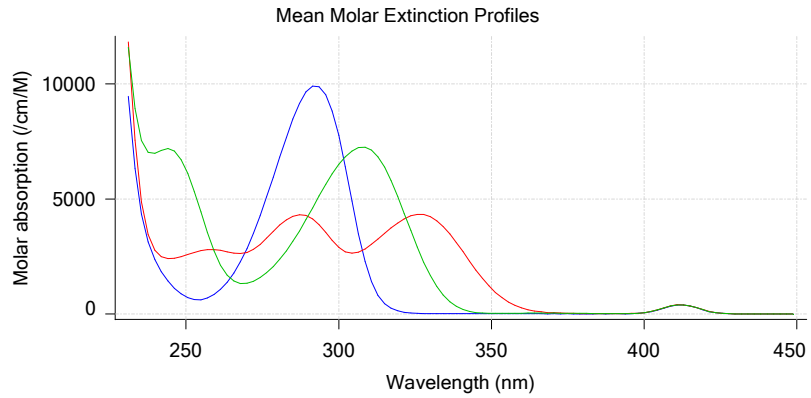
Component assay results

Titration	Methanol weight%	Direction	Result type	Dielectric constant	[H2O]	Ionic strength	Temperature	psKa 1	psKa 2
17I-12014 Points 4 to 37	49.62 %	Up	UV-metric pKa	56.5	24.6 M	0.157 M	25.0°C ☑	4.75 ☑	9.27
17I-12014 Points 39 to 79	39.80 %	Up	UV-metric pKa	61.1	30.1 M	0.166 M	25.0°C ☑	4.68 ☑	9.19
17I-12014 Points 81 to 128	29.84 %	Up	UV-metric pKa	65.6	36.0 M	0.173 M	25.0°C ☑	4.73 ☑	9.04

Graphs







UV-metric psKa Titration 1 of 3 17I-12014 Points 4 to 37

Results

pKa 1	**4.75**
pKa 2	**9.27**
RMSD	**0.055 0.045 0.032**
Chi squared	**0.2284**
PCA calculated number of pKas	**2**
Average ionic strength	**0.157 M**
Average temperature	**25.0°C**
Analyte concentration range	**30.5 µM to 28.8 µM**
Methanol weight %	**49.6 %**
Dielectric constant	**56.5**
Water concentration	**24.6 M**

Sample name: **Pyridoxine HCl**
Assay name: **UV-metric psKa**
Assay ID: **17I-12014**
Filename: **C:\Sirius_T3\Mehtap\20170912_test_comp_pKa_diffexp\17I-12014_Pyridoxine HCl_UV-metric psKa.t3r**

Experiment start time: **9/12/2017 7:57:21 PM**
Analyst: **Dorothy Levorse**
Instrument ID: **T311053**

Results (continued)

Number of pKas source **Predicted**
Wavelength clipping **230.0 nm to 450.0 nm**
pH clipping **1.469 to 12.530**

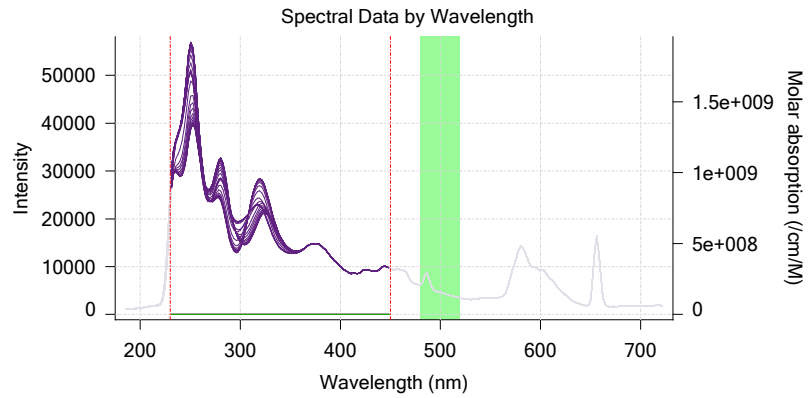
Warnings and errors

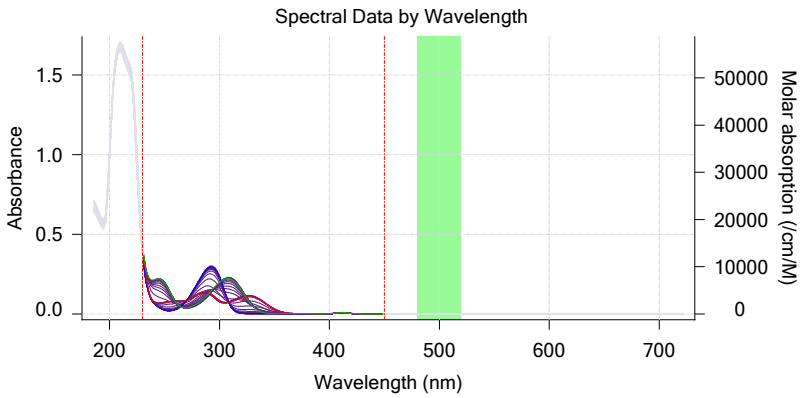
Errors None
Warnings RMSD exceeds warning threshold

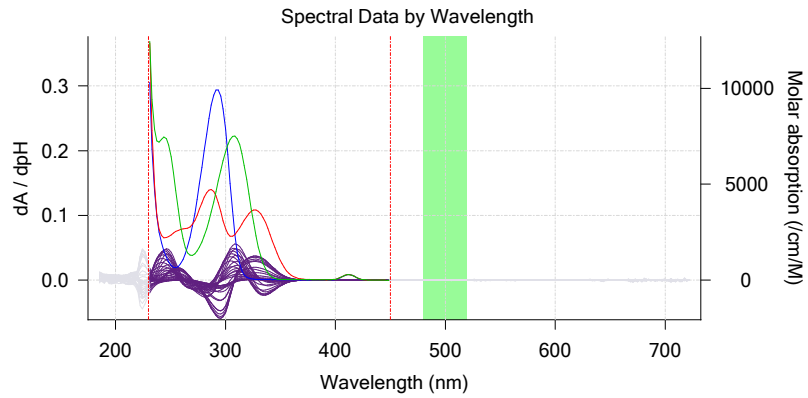
Assay Settings

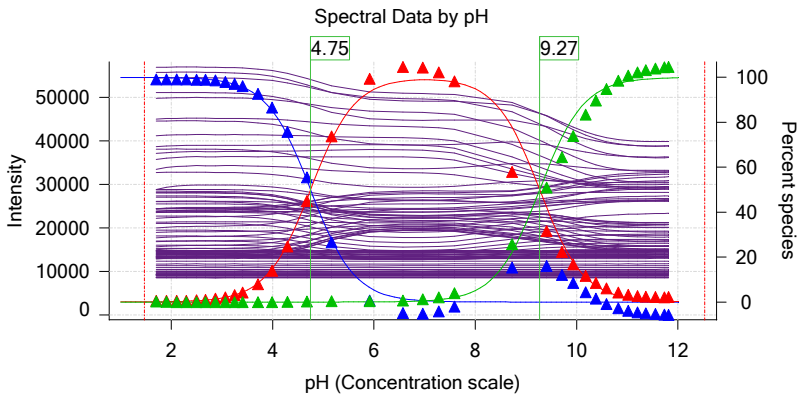
Setting	Value	Original Value	Date/Time changed	Imported from
Buffer in use	Yes			
Buffer type	Phosphate Buffer			
Assay Medium				
Volume of buffer introduced	0.025000 mL			
Add buffer manually	Manual			

Graphs





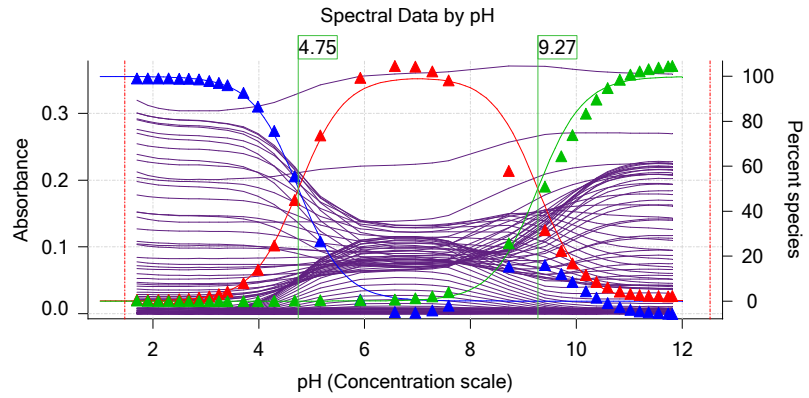


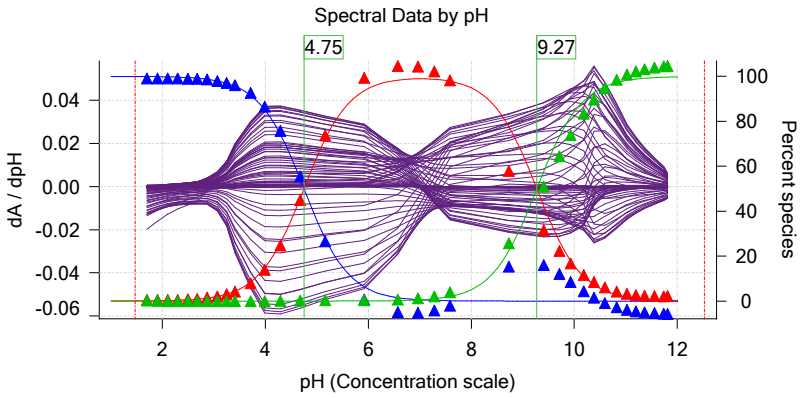


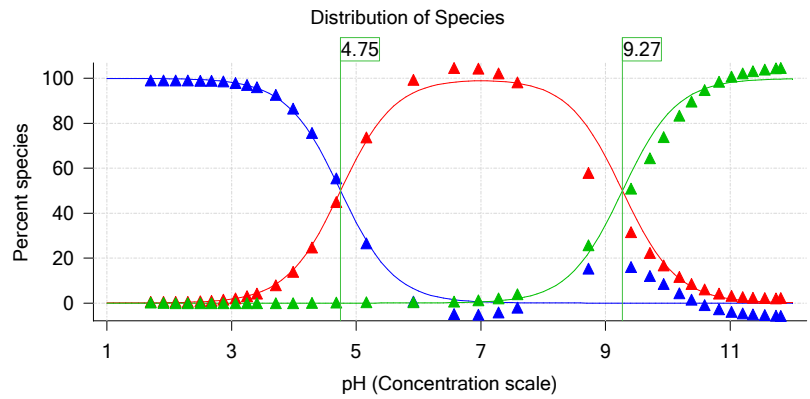
Sample name: **Pyridoxine HCl**
Assay name: **UV-metric psKa**
Assay ID: **17I-12014**
Filename: **C:\Sirius_T3\Mehtap\20170912_test_comp_pKa_diffexp\17I-12014_Pyridoxine HCl_UV-metric psKa.t3r**

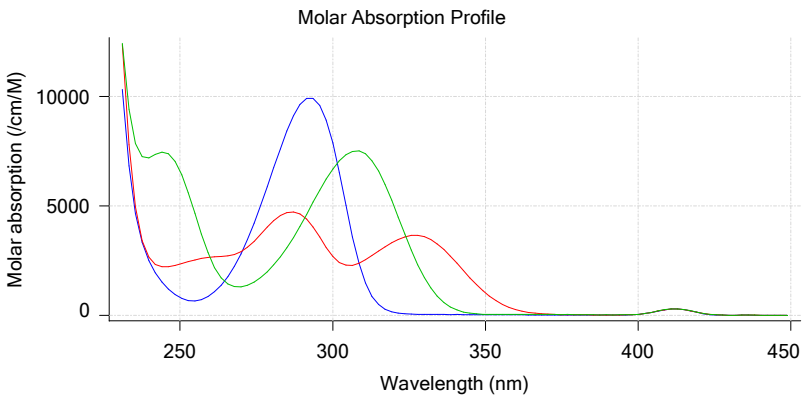
Experiment start time: **9/12/2017 7:57:21 PM**
Analyst: **Dorothy Levorse**
Instrument ID: **T311053**

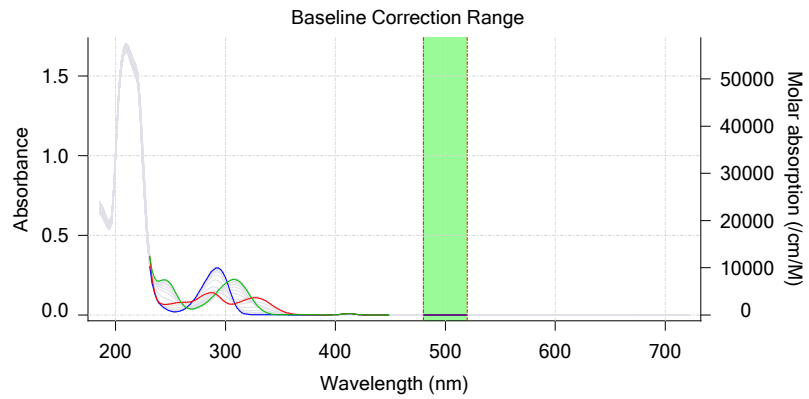
Graphs (continued)

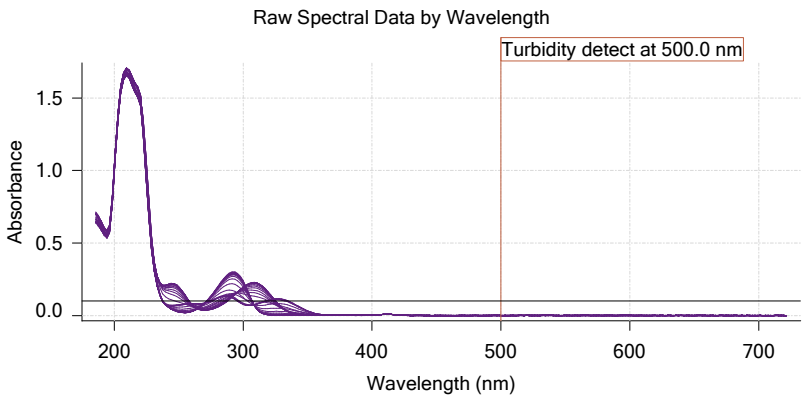












UV-metric psKa Titration 2 of 3 17I-12014 Points 39 to 79

Results

pKa 1	**4.68**
pKa 2	**9.19**
RMSD	**0.084 0.071 0.049**
Chi squared	**0.3942**
PCA calculated number of pKas	**3**
Average ionic strength	**0.166 M**
Average temperature	**25.0°C**
Analyte concentration range	**25.0 µM to 23.6 µM**
Methanol weight %	**39.8 %**
Dielectric constant	**61.1**
Water concentration	**30.1 M**

Sample name: **Pyridoxine HCl**
Assay name: **UV-metric psKa**
Assay ID: **17I-12014**
Filename: **C:\Sirius_T3\Mehtap\20170912_test_comp_pKa_diffexp\17I-12014_Pyridoxine HCl_UV-metric psKa.t3r**

Experiment start time: **9/12/2017 7:57:21 PM**
Analyst: **Dorothy Levorse**
Instrument ID: **T311053**

Results (continued)

Number of pKas source **Predicted**
Wavelength clipping **230.0 nm to 450.0 nm**
pH clipping **1.445 to 12.525**

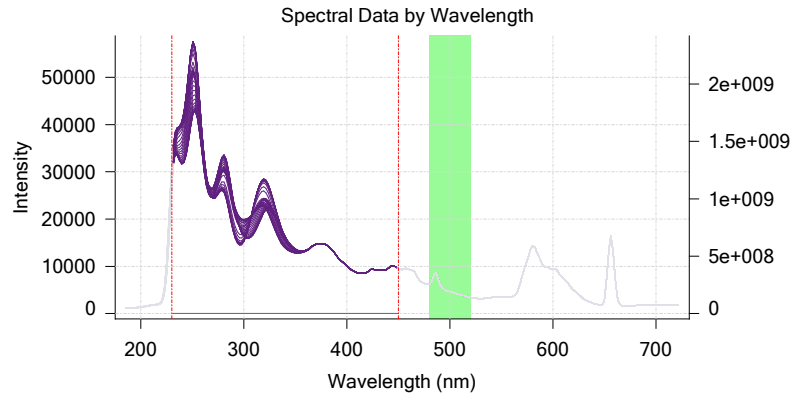
Warnings and errors

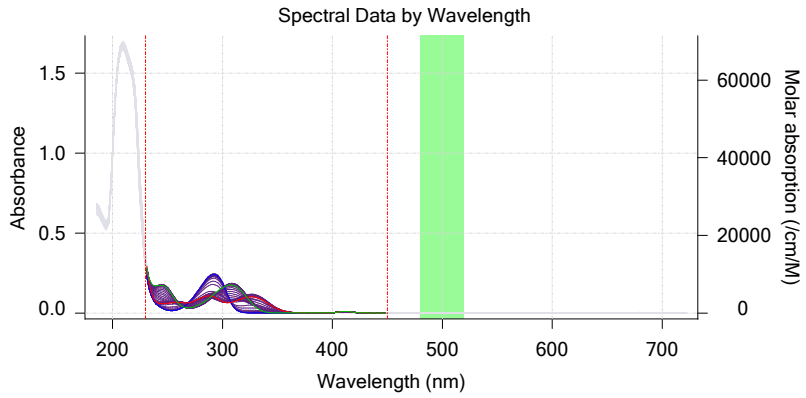
Errors None
Warnings RMSD exceeds warning threshold
PCA calculation disagrees with predicted number of pKas

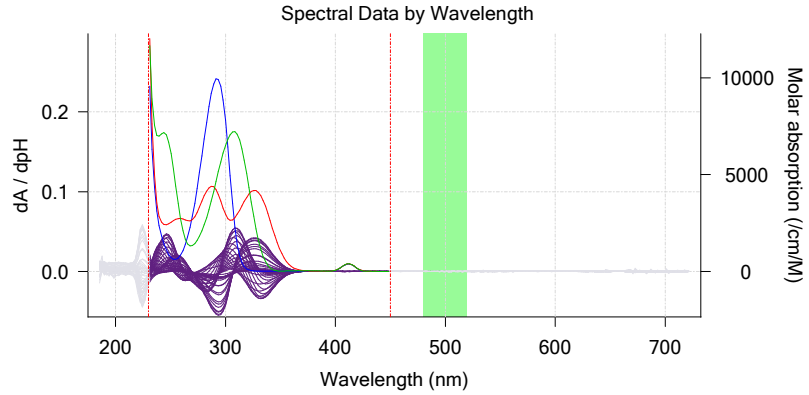
Assay Settings

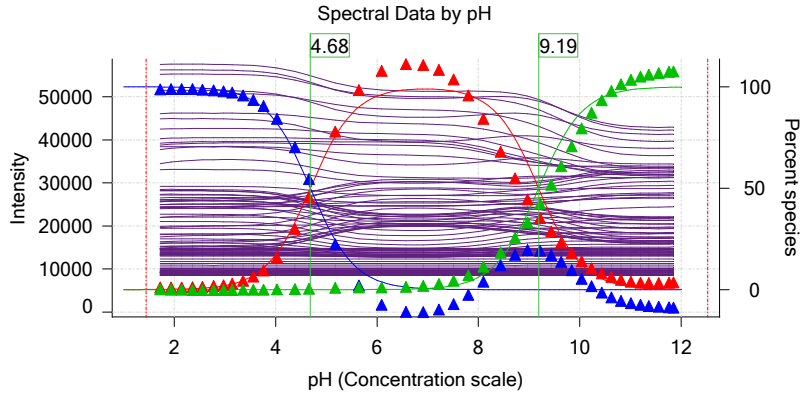
Setting	Value	Original Value	Date/Time changed	Imported from
Buffer in use	Yes			
Buffer type	Phosphate Buffer			
Assay Medium				
Volume of buffer introduced	0.025000 mL			
Add buffer manually	Manual			

Graphs





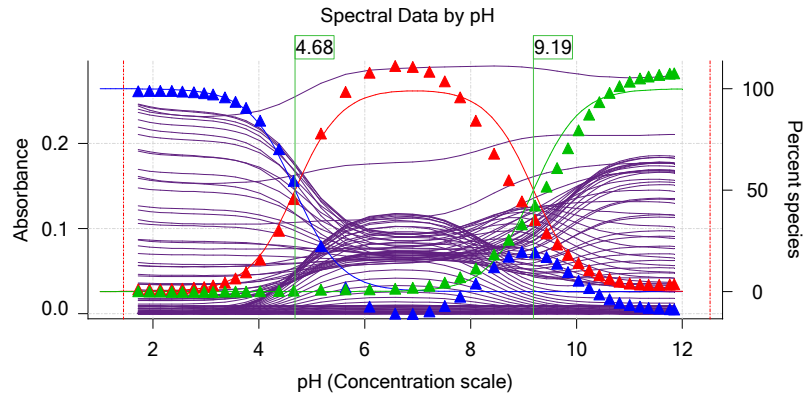


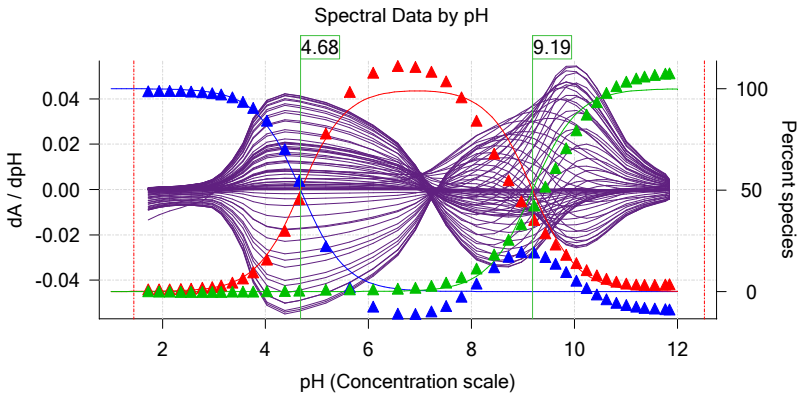


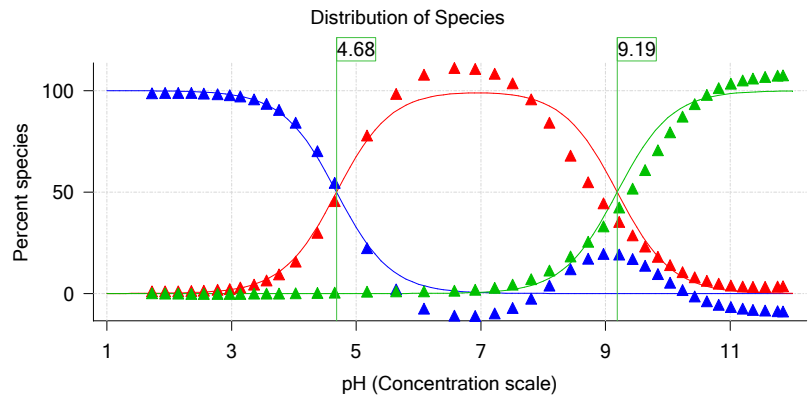
Sample name: **Pyridoxine HCl**
Assay name: **UV-metric psKa**
Assay ID: **17I-12014**
Filename: **C:\Sirius_T3\Mehtap\20170912_test_comp_pKa_diffexp\17I-12014_Pyridoxine HCl_UV-metric psKa.t3r**

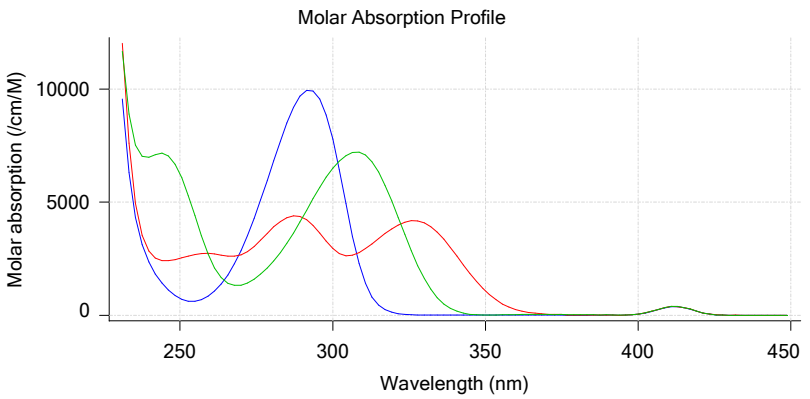
Experiment start time: **9/12/2017 7:57:21 PM**
Analyst: **Dorothy Levorse**
Instrument ID: **T311053**

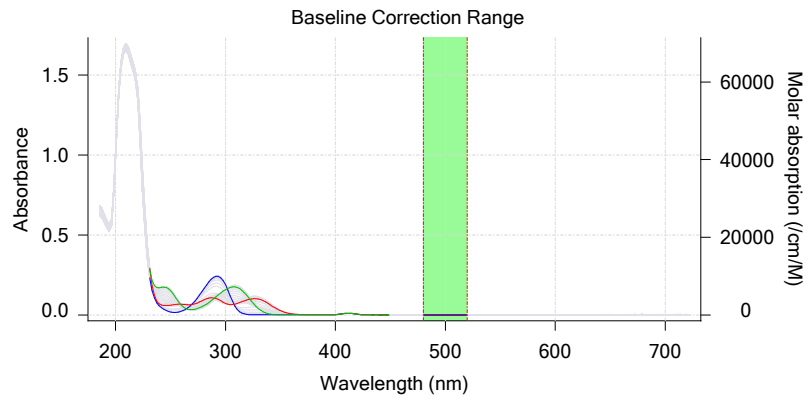
Graphs (continued)

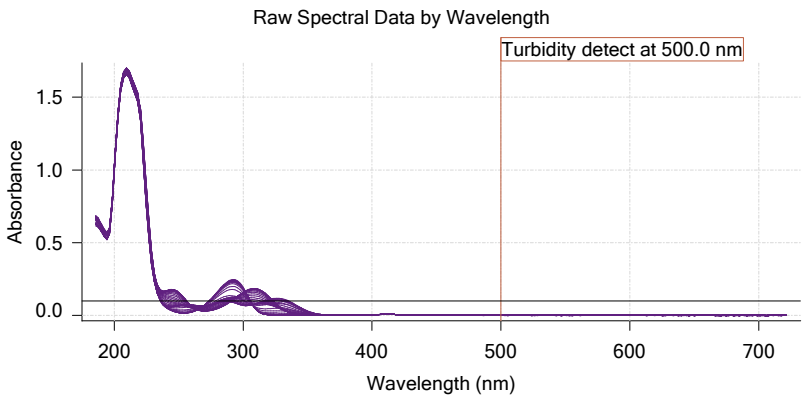












UV-metric psKa Titration 3 of 3 17I-12014 Points 81 to 128

Results

pKa 1	**4.73**
pKa 2	**9.04**
RMSD	**0.088 0.066 0.061**
Chi squared	**0.5032**
PCA calculated number of pKas	**4**
Average ionic strength	**0.173 M**
Average temperature	**25.0°C**
Analyte concentration range	**19.1 µM to 18.0 µM**
Methanol weight %	**29.8 %**
Dielectric constant	**65.6**
Water concentration	**36.0 M**

Sample name: **Pyridoxine HCl**
Assay name: **UV-metric psKa**
Assay ID: **17I-12014**
Filename: **C:\Sirius_T3\Mehtap\20170912_test_comp_pKa_diffexp\17I-12014_Pyridoxine HCl_UV-metric psKa.t3r**

Experiment start time: **9/12/2017 7:57:21 PM**
Analyst: **Dorothy Levorse**
Instrument ID: **T311053**

Results (continued)

Number of pKas source **Predicted**
Wavelength clipping **230.0 nm to 450.0 nm**
pH clipping **1.455 to 12.539**

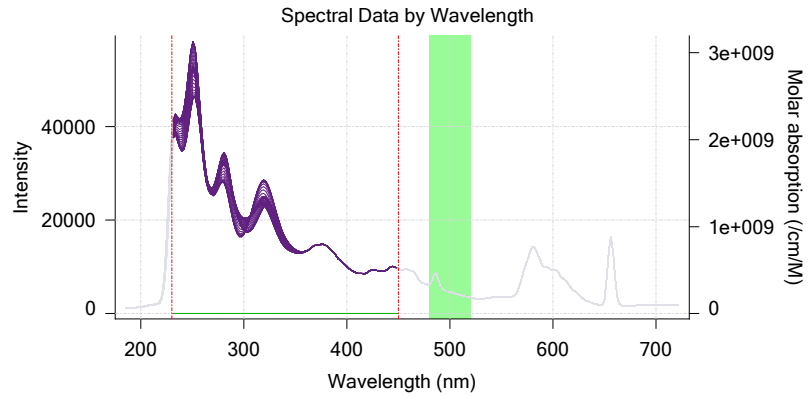
Warnings and errors

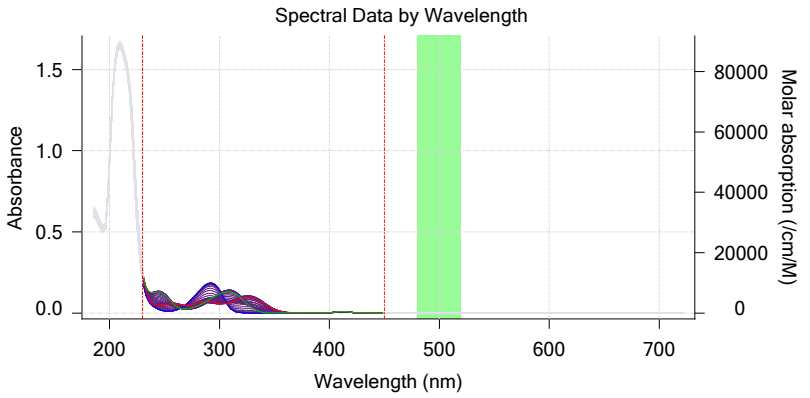
Errors None
Warnings RMSD exceeds warning threshold
PCA calculation disagrees with predicted number of pKas

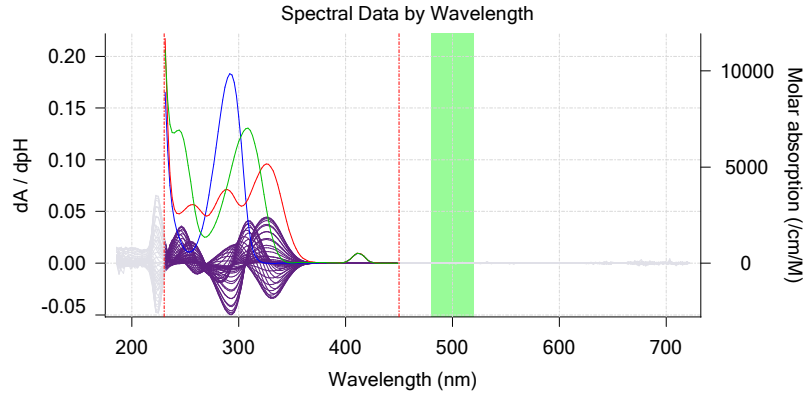
Assay Settings

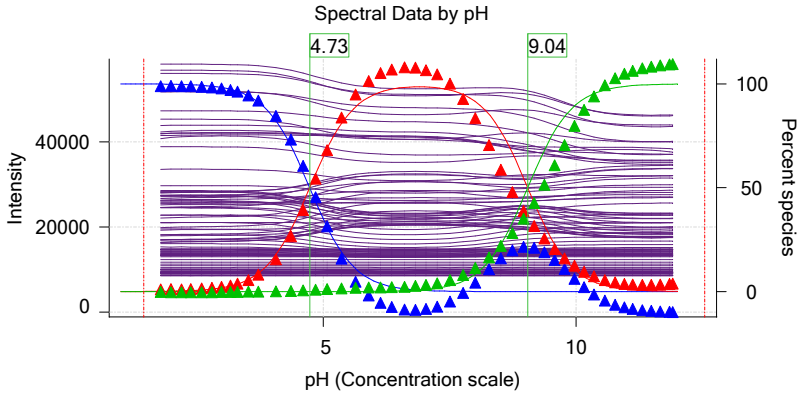
Setting	Value	Original Value	Date/Time changed	Imported from
Buffer in use	Yes			
Buffer type	Phosphate Buffer			
Assay Medium				
Volume of buffer introduced	0.025000 mL			
Add buffer manually	Manual			

Graphs





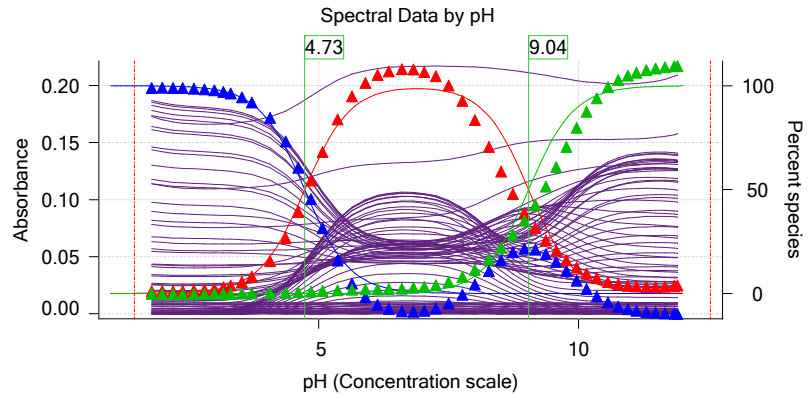


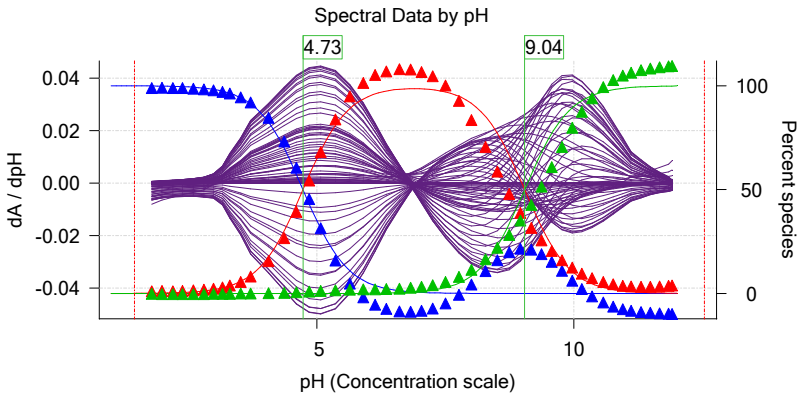


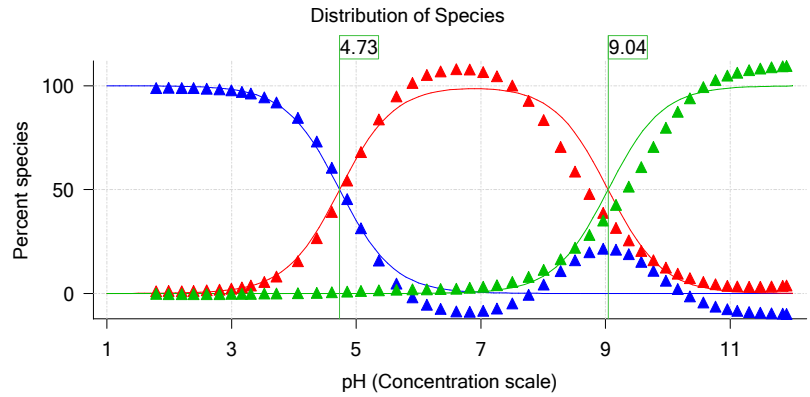
Sample name: **Pyridoxine HCl**
Assay name: **UV-metric psKa**
Assay ID: **17I-12014**
Filename: **C:\Sirius_T3\Mehtap\20170912_test_comp_pKa_diffexp\17I-12014_Pyridoxine HCl_UV-metric psKa.t3r**

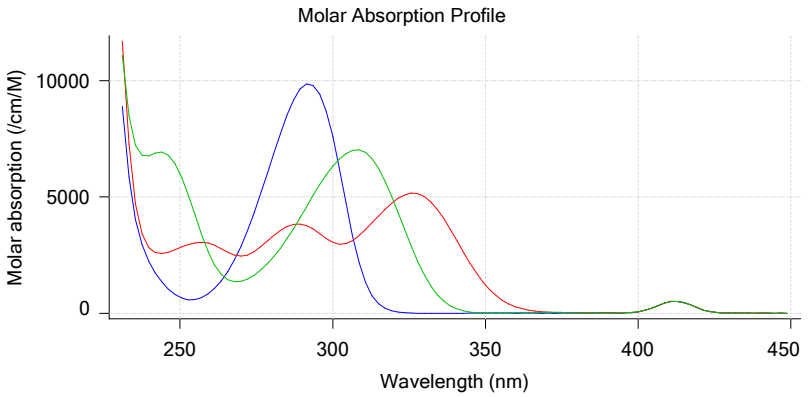
Experiment start time: **9/12/2017 7:57:21 PM**
Analyst: **Dorothy Levorse**
Instrument ID: **T311053**

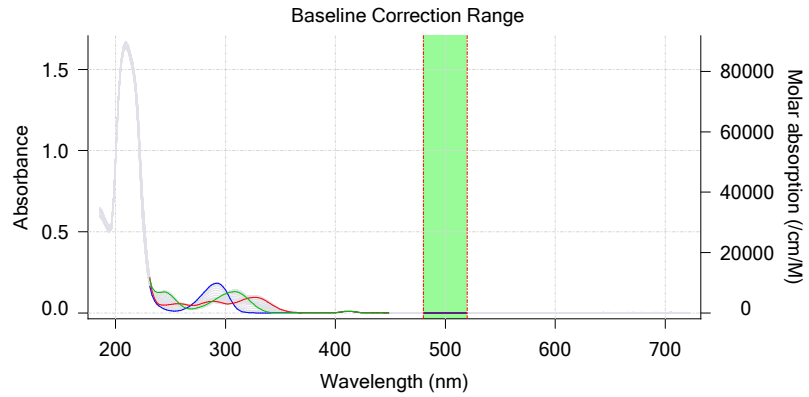
Graphs (continued)

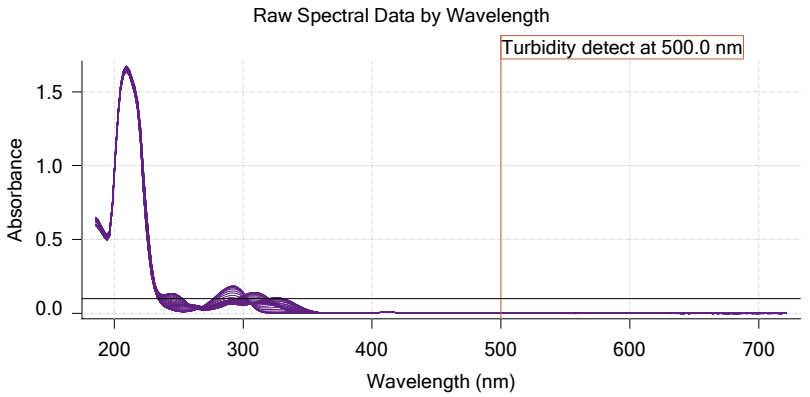












Assay Model

Settings	Value	Date/Time changed	Imported from
Sample name	Pyridoxine HCl	9/12/2017 1:25:48 PM	User entered value
Sample by	Volume		Default value
Sample volume	0.0010 mL	9/12/2017 1:22:51 PM	User entered value
Solvent	DMSO		Default value
Sample concentration	0.048630 M	9/12/2017 1:22:51 PM	User entered value
Solubility	Unknown		Default value
Molecular weight	205.64	9/12/2017 1:23:01 PM	User entered value
Individual pKa ionic environments	No		Default value
Number of pKas	2	9/12/2017 1:22:51 PM	User entered value
Sample is a	Ampholyte	9/12/2017 1:22:51 PM	User entered value
pKa 1	4.90	9/12/2017 1:22:51 PM	User entered value
Type	Base	9/12/2017 1:22:51 PM	User entered value
pKa 2	8.80	9/12/2017 1:22:51 PM	User entered value
Type	Acid	9/12/2017 1:22:51 PM	User entered value

Sample name: **Pyridoxine HCl**
Assay name: **UV-metric psKa**
Assay ID: **17I-12014**
Filename: **C:\Sirius_T3\Mehtap\20170912_test_comp_pKa_diffexp\17I-12014_Pyridoxine HCl_UV-metric psKa.t3r**

Experiment start time: **9/12/2017 7:57:21 PM**
Analyst: **Dorothy Levorse**
Instrument ID: **T311053**

Assay Model (continued)

Settings	Value	Date/Time changed	Imported from
logp (XH2 +)	-10.00		Default value
logP (neutral XH)	-10.00	9/12/2017 1:22:51 PM	User entered value
logP (X -)	-10.00		Default value
Stoichiometry	1.00000		Default value
Aprotic counterion name	Chloride		From standards.xml file
Stoichiometry	1.00		From standards.xml file
Charge per counterion	-1		From standards.xml file

Assay Settings

Setting	Value	Original Value	Date/Time changed	Imported from
General Settings				
Analyst name	Dorothy Levorse			
Separate reference vial	Yes			
Standard Experiment Settings				
Number of titrations	3			
Minimum pH	2.000			
Maximum pH	12.000			
pH step between points of	0.200			
Minimum titrant addition	0.00002 mL			
Maximum titrant addition	0.10000 mL			
Argon flow rate	100%			
Start titration using	Cautious pH adjust			
Advanced General Settings				
Detect turbidity using	Spectrometer			
Monitor at a wavelength of	500.0 nm			
Absorbance threshold of	0.100			
Collect turbidity sensor data	No			
Stir after titrant addition for	5 seconds			
For titrant addition, stir at	15%			
Titrant Pre-Dose				
Titrant pre-dose	None			
Assay Medium				
Cosolvent in use	Yes			
Cosolvent type	Methanol			
Cosolvent volume	1.15 mL			
Cosolvent added	Automatic			
ISA water volume	0.35 mL			
Water added	Automatic			
After water addition, stir for	5 seconds			
At a speed of	15%			
Buffer in use	Yes			
Buffer type	Phosphate Buffer			
Volume of buffer introduced	0.025000 mL			
Add buffer manually	Manual			
After medium addition, stir for	5 seconds			
Sample Sonication				
Sonicate	No			
Sample Dissolution				
Perform a dissolution stage	No			
Carbonate purge				
Perform a carbonate purge	No			
Temperature Control				
Wait for temperature	Yes			
Required start temperature	25.0°C			
Acceptable deviation	0.5°C			
Time to wait	60 seconds			
Stir speed of	15%			

Sample name: **Pyridoxine HCl**
Assay name: **UV-metric psKa**
Assay ID: **17I-12014**
Filename: **C:\Sirius_T3\Mehtap\20170912_test_comp_pKa_diffexp\17I-12014_Pyridoxine HCl_UV-metric psKa.t3r**
Experiment start time: **9/12/2017 7:57:21 PM**
Analyst: **Dorothy Levorse**
Instrument ID: **T311053**

Assay Settings (continued)

Setting	Value	Original Value	Date/Time changed	Imported from
Titration 1				
Titrate from	Low to high pH			
Adjust to start pH	Yes			
After pH adjust stir for	10 seconds			
Titration 2				
Titrate from	Low to high pH			
Additional cosolvent volume	0.00 mL			
Add additional water	0.15 mL			
Additional water added	Automatic			
After pH adjust stir for	10 seconds			
Titration 3				
Titrate from	Low to high pH			
Additional cosolvent volume	0.00 mL			
Add additional water	0.34 mL			
Additional water added	Automatic			
After pH adjust stir for	10 seconds			
Data Point Stability				
Stir during data point collection	Yes			
For point collection, stir at	15%			
Delay before data point collection	0 seconds			
Number of points to average	20 points			
Time interval between points	0.50 seconds			
Required maximum standard deviation	0.00500 dpH/dt			
Stability timeout after	60 seconds			
Experiment cleanup				
Adjust pH to cleanup	To start pH			
And then stir for	60 seconds			
For cleaning, stir at	20%			
Then add water volume	0.25 mL			
And then stir for	30 seconds			

Calibration Settings

Setting	Value	Date/Time changed	Imported from
Four-Plus alpha	0.080	9/12/2017 7:57:21 PM	C:\Sirius_T3\HCl17I12.t3r
Four-Plus S	1.0027	9/12/2017 7:57:21 PM	C:\Sirius_T3\HCl17I12.t3r
Four-Plus jH	1.2	9/12/2017 7:57:21 PM	C:\Sirius_T3\HCl17I12.t3r
Four-Plus jOH	-0.6	9/12/2017 7:57:21 PM	C:\Sirius_T3\HCl17I12.t3r
Base concentration factor	1.000		Default value
Acid concentration factor	1.000	9/12/2017 7:57:21 PM	C:\Sirius_T3\HCl17I12.t3r

Instrument Settings

Setting	Value	Batch Id	Install date
Instrument owner	Merck		
Instrument ID	T311053		
Instrument type	T3 Simulator		
Software version	1.1.3.0		
Dispenser module		T3DM1100253	3/31/2009 6:24:52 AM
Dispenser 0	Water		3/31/2009 6:25:05 AM
Syringe volume	2.5 mL		
Firmware version	1.2.1(r2)		
Titrant	Water (0.15 M KCl)	8-18-17	9/8/2017 9:22:43 AM
Dispenser 2	Acid		3/31/2009 6:25:11 AM
Syringe volume	0.5 mL		
Firmware version	1.2.1(r2)		
Titrant	Acid (0.5 M HCl)	166940	9/8/2017 9:21:27 AM
Dispenser 1	Base		3/31/2009 6:25:21 AM

Sample name: **Pyridoxine HCl**
Assay name: **UV-metric psKa**
Assay ID: **17I-12014**
Filename: **C:\Sirius_T3\Mehtap\20170912_test_comp_pKa_diffexp\17I-12014_Pyridoxine HCl_UV-metric psKa.t3r**

Experiment start time: **9/12/2017 7:57:21 PM**
Analyst: **Dorothy Levorse**
Instrument ID: **T311053**

Instrument Settings (continued)

Setting	Value	Batch Id	Install date
Syringe volume	0.5 mL		
Firmware version	1.2.1(r2)		
Titrant	Base (0.5 M KOH)	01/06/17	9/8/2017 9:20:03 AM
Dispenser 5	Cosolvent		3/31/2009 6:26:24 AM
Syringe volume	2.5 mL		
Firmware version	1.2.1(r2)		
Distribution valve 5	Distribution Valve		3/31/2009 6:28:19 AM
Firmware version	1.1.3		
Port A	Methanol (80%, 0.15 M KCl)	8-15-17	9/7/2017 3:40:52 PM
Dispenser 3	Buffer		8/3/2010 6:05:16 AM
Syringe volume	0.5 mL		
Firmware version	1.2.1(r2)		
Titrant	Phosphate Buffer		9/12/2017 12:32:29 PM
Dispenser 6	Octanol		10/22/2010 11:52:43 AM
Syringe volume	0.5 mL		
Firmware version	1.2.1(r2)		
Titrant	Octanol		9/12/2017 12:32:22 PM
Titrator		T3TM1100153	3/31/2009 6:24:17 AM
Horizontal axis firmware version	1.17 AI1DI2DO2 Stepper 2		
Vertical axis firmware version	1.17 AI1DI2DO2 Stepper 2		
Chassis I/O firmware version	1.11 AI1DI0DO4 Norgren I/O		
Probe I/O firmware version	1.1.1		
Electrode	T3 Electrode	T3E0769	8/15/2017 10:21:54 AM
E0 calibration	-7.42 mV		9/12/2017 7:57:51 PM
Filling solution	3M KCl	KCL095	9/10/2017 10:38:19 AM
Liquids			
Wash 1	50% IPA:50% Water		9/12/2017 9:11:25 AM
Wash 2	0.5% Trition X-100 in H20		9/12/2017 9:11:30 AM
Buffer position 1	pH7 Wash		9/12/2017 9:11:33 AM
Buffer position 2	pH 7		9/12/2017 9:11:37 AM
Storage position			9/12/2017 9:11:42 AM
Wash water	8.6e+003 mL	9-11-17	9/11/2017 4:28:43 PM
Waste	1.5e+003 mL		9/11/2017 4:28:49 PM
Temperature controller			8/5/2010 7:35:13 AM
Turbidity detector			3/31/2009 6:24:45 AM
Spectrometer		072390	11/23/2010 12:22:28 PM
Dip probe		11086	
Wavelength coefficient A0	185.563		
Wavelength coefficient A1	2.17439		
Wavelength coefficient A2	-0.000285622		
Total lamp lit time	75:06:25		11/23/2010 12:22:28 PM
Calibrated on	9/6/2017 9:33:02 AM		
Integration time	11		
Scans averaged	10		
Autoloader		T3AL1100237	11/10/2015 10:34:13 AM
Left-right axis firmware version	1.17 AI1DI2DO2 Stepper 2		
Front-back axis firmware version	1.17 AI1DI2DO2 Stepper 2		
Vertical axis firmware version	1.17 AI1DI2DO2 Stepper 2		
Chassis I/O firmware version	1.11 AI1DI0DO4 Norgren I/O		
Configuration			
Alternate titration position	Titration position		
Alternate reference position	Reference position		
Maximum standard vial volume	3.50 mL		
Maximum alternate vial volume	25.00 mL		
Automatic action idle period	5 minute(s)		
Titrant tube volume	1.3 mL		
Syringe flush count	3.50		
Flowing wash pump volume	20.0 mL		

Sample name: **Pyridoxine HCl**
Assay name: **UV-metric psKa**
Assay ID: **17I-12014**
Filename: **C:\Sirius_T3\Mehtap\20170912_test_comp_pKa_diffexp\17I-12014_Pyridoxine HCl_UV-metric psKa.t3r**

Experiment start time: **9/12/2017 7:57:21 PM**
Analyst: **Dorothy Levorse**
Instrument ID: **T311053**

Instrument Settings (continued)

Setting	Value	Batch Id	Install date
Flowing wash stir duration	5 s		
Flowing wash stir speed	30%		
Solvent wash stir duration	5 s		
Solvent wash stir speed	30%		
Surfactant wash stir duration	5 s		
Surfactant wash stir speed	30%		
E0 calibration minimum number of points	10		
E0 calibration maximum standard deviation	0.01500		
E0 calibration timeout period	60 s		
E0 calibration stir duration	5 s		
E0 calibration preparation stir speed	30%		
E0 calibration buffer wash stir duration	5 s		
E0 calibration buffer wash stir speed	30%		
E0 calibration reading stir speed	0%		
Spectrometer calibration stir duration	5 s		
Spectrometer calibration stir speed	30%		
Spectrometer calibration wash pump volume	20.0 mL		
Spectrometer calibration wash stir duration	5 s		
Spectrometer calibration wash stir speed	30%		
Overhead dispense height	10000		

Refinement Settings

Setting	Value	Default value
Turbidity detection method	Spectrometer	Spectrometer
Turbidity wavelength to assess	500.0 nm	500.0 nm
Turbidity maximum absorbance	0.100	0.100
Turbidity probe threshold	50.00	50.00
Exclude turbid points	Yes	Yes
Low intensity warning threshold	100	100
Minimum absorbance change threshold	0.100	0.100
Eigenvector autocorrelation threshold	0.80	0.80
Maximum RMSD severe warning	0.250	0.250
Maximum RMSD warning	0.050	0.050